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Electrophoretic Pattern of Alcohol and Acid Induced Proteins of *Saccharomyces cerevisiae*

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Gene expression of organisms is induced by various environmental factors. Protein has the ability to stabilize the organism against environmental conditions. Proteins are induced by acid or alkali during long term aerobic or anaerobic condition in the growth medium. In our study *Saccharomyces cerevisiae* are treated with acid and alcohol for the expression of proteins the express proteins are analysed by SDS – Poly Acrylamide Gel Electrophorosis.

Keywords: *Saccharomyces cerevisiae,* acid, alkali, SDS - PAGE.

Yeast is ubiquitous unicellular fungi and hence eukaryotic microorganisms. They live as saprophytes on plants or animal material, where they catabolize preferentially sugars but also polyols, alcohols, organic acids and aminoacids as sources for carbon and energy. Yeast notably *Saccharomyces cerevisiae* are worthy of special attention, due to their major contribution in industrial. The yeast exhibit dimorphism changes from one form to other are often influenced by the preailing nutritional condition. Yeast cell rapidly stimulate a mitogen activated protein (MAP) kinase cascade, the high osmolarity glycerol (HOG) pathway. Accumulation of the osmolyte glycerol, which is also controlled by altering transmembrane glycerol transport, yeasts are exposed to highly variable environment with respect to the availability and quality of nutrients, temperature, pH, radiation, access to oxygen. Yeasts cell have developed mechanisms to adjust with in certain limits, to high external osmolarity.

For the preservation of the food, weak organic acids are used as food preservatives to inhibit the growth of spoilage Yeast, including Saccharomyces *cerevisiae* long-term adaptation to weak organic acids requires the increased expression of those proteins depends on nutrient sources and other parameters. In yeast growing on glucose the doubling time about two hours, because the ATP pool of 4mm/gm of dry weight (Werliel *et al.,* 1974) would be exhausted in about dix seconds if protein synthesis continued at tis normal rate of 0.16mM of aminoacids polymerized per second gram of dry weight (Waldron and Lacroute, 1975). Yeast produces several polyhydric alcohols, including glycerol, arbitol, erythritol, mannitol and xylitol. Lacticacid and Formic acid are used for food preservatives. The ability of yeast to grow in the presence of weak organic acid and other alcohols is an important cause of food spoilage. As a result, the yeast is forced to generate an alternate route for resistant through the expression of an adaptive proteins.

In this study, our aim is to detect the adaptive enzyme (protein) of yeast by exposure to lactic acid, citric acid ethanol, propanol and butanol.

MATERIAL AND METHODS

Isolation of *Saccharmyces cerevisiae*

Infected grapes was collected from the market .Crush the grapes and prepare the juice. One loopful of sample was streaked on Yeast Peptone Agar (YPD) , which provides a selective medium for the isolation of *Saccharomyces cerevisiae* .The isolated culture was then incubated

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at 30^0 C for 4-5 days and confirmed as *Saccharomyces cerevisiae* by staining technique .

Kinetics of cell growth in different chemicals

Saccharomyces cerevisiae was grown on YPD medium with various chemical such as Citric acid, Lactic acid, Ethanol, Propanol, Butanol; Ethanol.1% concentration of above chemicals was added in different flasks. One ml of 24hrs culture was inoculated into the medium and incubated at 30 ^{0}C. Measure the optical density of culture at 1hr interval for 24hrs.

Expression of protein

Prepared Yeast peptone broth in five different flasks .1% of Citric acid, Lactic acid, Propanol, Butanol, Ethanol chemicals was added in different flasks. All the flasks were incubated at 30 ^{0}C for 24hrs for the expression of protein.

Preparation of protein

The culture samples were centrifuged at 10,000rpm for ten minutes, the pellets were resuspended in Tris-HCl(pH-7) buffer and grind well. The sample was centrifuged at 10,000rpm for two minutes. The supernatant was collected. The protein was precipitated with 10% TCA. The protein was collected and precipitated by centrifugation at 10,000rpm for ten minutes at 4^0C. Then washed with one ml of 80% acetone. Centrifuged and the pellet was dissolved in 50µl of Tris-HCl, buffer pH-7.

RESULTS AND DISCUSSION

The results of this study showed that the chemical resistance of *Saccharomyces cerevisiae* is a complex process which is also related to heat shock and general stress response. *Saccharomyces cerevisiae* was grown in heavily buffered rich broth, a condition which enables growth to greater extremes of pH. Cultures were grown for several generations under stress condition designed to force *Saccharomyces cerevisiae* to grow at the extreme limits of tolerances of pH, aerobically or anaerobically. Maximum number of colonies was occurred in the yeast treated with Citric acid, Lactic acid, Ethanol than Butanol, Propanol. SDS-PAGE of five different chemicals treated and control yeast strains protein extracts were studied (Plate -1). In the coomassive blue staining protein patterns containing up to 24 distinct bands, with this one major band was observed. The proteins ranged in molecular weight from 160KDa to 25KDa in 10% separating gels. Alcohol treated yeast strains produced a characteristic protein band was easily identified in the position of (45KDa) in the gel.

But in Lactic acid and Citric acid treated yeast strain produced protein band was observed in the molecular weight of 47KDa. 47KDa stress protein was produced by the yeast treated with Lactic acid and Citric acid. Proteins induced by

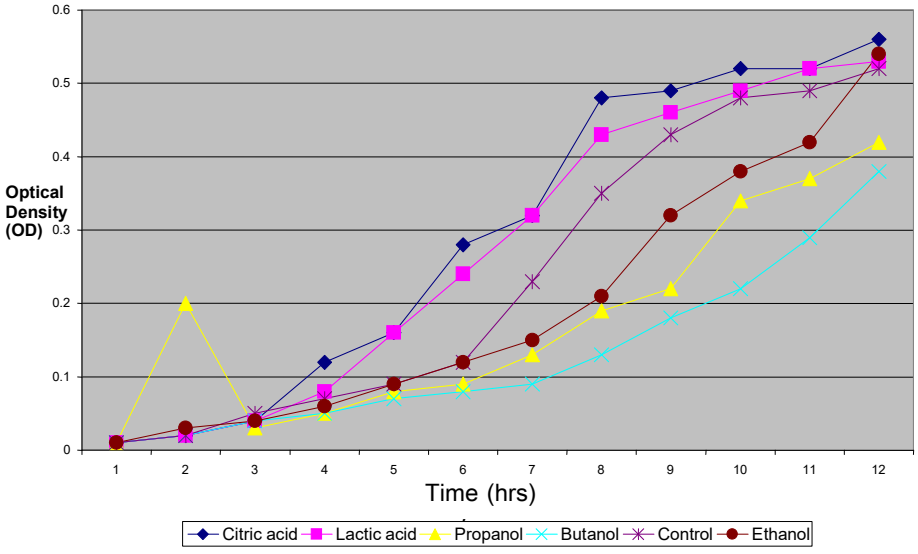

Fig. 1. Growth kinetics of Saccharomyces cerecisiae in different chemicals

acid or base during long term aerobic or anaerobic growth in Yeast peptone medium, were identified in *Saccharomyces cerevisiae*. During the treatment of microbes by chemicals, various proteins molecules are synthesized. The amount of protein and nature of protein are varies depend on the nature of the chemicals. The glutamate decarboxylase system neutralizes acidity and enhances the survival of organisms in extreme acids, its induction during anaerobic growth may protect in alkaline grown cells from the acidification resulting from anaerobic fermentation (Blankenhorn *et al.,* 1999). The survival and growth of Yeast with various chemical stresses were observed in Fig. 1. From this result, we found that citric acid, lactic acid and ethanol are induced chemical stress protein at low pH when the yeast is growing from the log phases. Because the yeast is secreted secondary metabolites like ethanol, citric acid lactic acid in the medium and it also stress to the yeast for synthesize a stress protein.

The pyruvate formatelyase was induced to higher levels at pH4.4 and induced two fold by propionate at pH6.The increase of alkyl hydroperoxide reductase (AhpC) in acid may help protect the cell from the greater concentration of oxidizing intermediates at low pH. The effect of pH, gene expression are known to interest with a number of other environmental factors most notably oxygenation .For example, the acidic induction of aminoacid decarboxylase is increased by anaerobiosis (Meng, *et al.,* 1992 and Neely, *et al.,* 1994), whereas the oxygen –induced cytochrome O is expressed by acid (Cotter, *et al.,* 1990). The SDS-poly acrylamide gel electrophoresis (PAGE) to elucidate patterns of protein response against various chemicals was shown in plate 1. The expressed proteins are same in citric acid and lactic acid treatment. The molecular weight of expressed protein is 47 KDa.

In Alkaline induction, expressed the GadA during anaerobic growth. Several amino acid decarboxylase systems are induced by acid and anaerobiosis (Auger *et al.,* 1989) and have been implicated in acid resistance (Lin *et al.,* 1995 and Waterman *et al.,* 1996). These systems involve a decarboxylase and a transporter for the substrate and/or product of decarboxylase (Neely

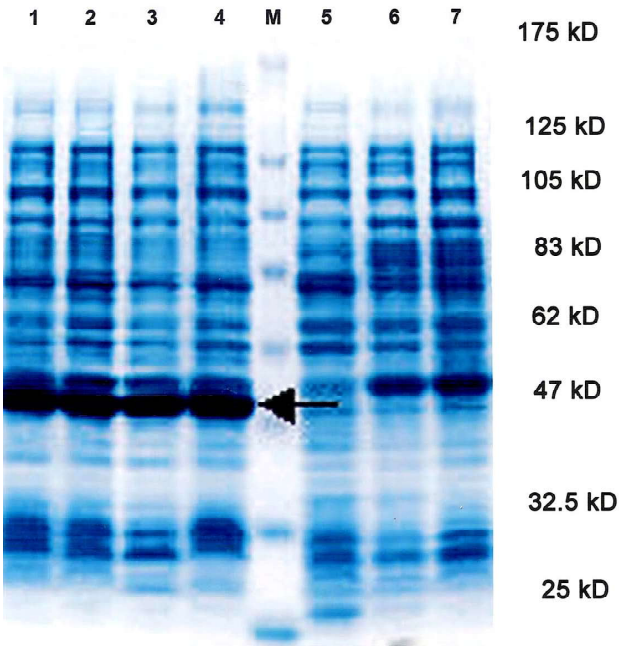

Plate 1. Electrophoretic pattern of alcohol and acid induced proteins of *S. cerevisiae*

Note: Lane 1 and 2 - Butanol; Lane 3 - Propanol; Lane 4 - Ethanol; M - Molecular weight Marker; Lane 5 - Control; Lane 6 - Lactic acid and Lane 7 - Citric acid

*et al.,*1994 and Small *et al.,* 1994). The putative transporter GadC for the glutamate system is required for extreme acid resistance of *E.coli* culture grown to stationary phase in base but is not essential for cultures grown in moderate acid (Hersh *et al.,* 1996).The glutamate system includes two homologous but unlinked glutamate decarboxylases ,GadA and GadB (Smith *et al.,* 1992);one of these ,GadB, is cotranscribed with GadC (Waterman *et al.,* 1996).

The homologous enzyme GadB has a isoelectric point and a molecular weight close to those of GadA. In some gels, a spot adjacent to GadA appeared to be induced similarity; this could be GadB, or it could be a secondary spot for GadA. Other decarboxylases, particularly for lysine (CadA) and arginine (Adi), would be expected to appear induced by acid. These proteins were not clearly separated in our gels, presumably because of their higher molecular weight (above 80,000). A protein induced by either acid or base, compared to pH-7, was isocitrate lyase (AceA). AceA is a glyoxylate shunt enzyme required for growth on acetate or fattyacids and induced by oxygen (Cronan *et al.,* 1992; Neely *et al.,* 1994). Thus it was surprising to find the highest levels of GadA during anaerobic growth in base. The glutamate system in particular contributes to survival of *Escherichia coli* at the most extreme low pH (Lin *et al.,* 1995; Smith, *et al.,* 1992 and Wateman, *et al.,* 1996).

In our study revealed that the soluble protein must reflect the physiological state of the cell, rather than morphological structure. Some variation in the protein patterns (Chemical stress protein) obtained depending on the physiological state of the cell.

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